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Resveratrol in combination with Green Tea reduces myocardial infarct area and improves histological changes in isoproterenol-induced cardiotoxic rats

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ABSTRACT

Background: Cardiovascular problem is a growing menace which is affecting people globally. Isoproterenol creates oxidative stress that leads to myocardial infarction. The extent of infarcted area in heart reflects the severity of toxicity. Histopathological changes induced by Isoproterenol become another tool for evaluating the effectiveness of the test drugs. In this study we therefore have considered these parameters to evaluate the effectiveness of our test drugs resveratrol, green tea and their combination. **Methods:** Thirty animals (n=5) were taken and randomly divided into Control, Isoproterenol (ISO), Resveratrol (RES) + ISO, Green Tea Extract (GTE) + ISO, RES + GTE+ ISO and RES + GTE groups. Rats were treated with the test drugs for thirty days and with ISO on 29th and 30th day. Animals were sacrificed on 31st day and histopathological studies and infarct size calculations were performed. **Results and Discussions:** Myofibril disintegration, vacuolation and pyknotic nucleus were observed in ISO treated rats which were considerably reversed by resveratrol, green tea extract and by their combination. The size of infarcted area in the heart of ISO treated rats was also significantly reduced when the rats were treated with these test drugs. **Conclusion:** Resveratrol and green tea extract both showed cardio protection in terms of restoration of histological changes and reduction of infarcted area but their combination was found to be better than them.

KEY WORDS: Myocardial infarction, Isoproterenol, histopathology, infarct size, resveratrol, green tea extract.

1. INTRODUCTION

Myocardial infarction (MI) or acute myocardial infarction (AMI) due to ischemic condition translates into heart attack. It occurs when the blood supply is interrupted to some part of the hearth due to blockage or occlusion of the artery. As the blood supply is curtailed, oxygen supply is interrupted causing necrosis and infarction of the myocardium. If this acute situation continues, death may occur [1]. In almost 64% of the cases silent heart attack is seen with no chest pain or other symptoms [2] leading to irreversible myocardial cell damage[3]. MI still remains the main causes of pathological issues and deaths globally [4].

1.1. Isoproterenol

Isoproterenol is a synthetic non-selective β-adrenoceptor agonist. It is a catecholamine which causes severe oxidative stress in the myocardium and lead to necrosis of the heart muscle [5]. The isoproterenol- (ISO) model of rat for studying myocardial necrosis is a standardized model being used to evaluate various natural and synthetic drugs for their cardioprotective potential [6,7]. It is a widely used experimental model because of technical simplicity, reproducibility, low mortality and resemblance to humans metabolic and morphological damages in heart [8, 9].

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1.2. Green tea (Camellia sinensis)

Tea is the most commonly consumed beverages throughout the world. It is extracted from the unfermented and dried leaves that contain highest concentration of polyphenol, a powerful antioxidants, which fights against the damage causing free radicals and strengthens the body defense system [10]. *Depending on the level of oxidation, green tea can be categorized into three different types;* (i) green tea (non-oxidized), (ii) oolong tea (partially oxidized) and (iii) black tea (oxidized)[11]. Green tea also possess cardioprotective [12], anti-inflammatory [13], anti-diabetic [14], and anti-bacterial activity [15].

1.3. Resveratrol

Resveratrol (3, 4, 5 trihydroxy trans stilbene) is a natural polyphenol, which is found in grapes, mulberries etc., and is traditionally used as medicinal herb. Resveratrol has been shown to possess

anticancer [16], cardioprotective [17, 18], neuroprotective [19], antidepressant[20] and antioxidant activity [18]. Resveratrol acts as a free radical-scavenger which boosts up the body defense mechanism by negating the damaging effect of free radicals.

2. MATERIALS AND METHODS

The experimental study was carried out under the standard laboratory conditions using adult Wistar albino rats of female sex (150 – 250 g), procured from Central Animal House Facility of Hamdard University, New Delhi. The rats were maintained on normal animal diet and water *ad libitum*. Protocol was approved by the Institutional Animal Ethics Committee of Hamdard University, New Delhi. Animals were divided into six groups; each groups consisting five animals. The animal groups and their treatment schedules were as follow:

Sl. No.	Groups	No. of rats	Drug/Supplement Given
1	**CONTROL**	5	Normal saline 2 ml/kg orally once a day for 30 days + normal saline 0.5 ml subcutaneous on 29th and 30th days
2	**ISO**	5	Normal saline 2 ml/kg orally once a day for 30 days + subcutaneous injection of isoproterenol 85 mg/kg on 29th and 30th days
3	**RES + ISO**	5	20 mg/kg resveratrol once a day for 30 days + subcutaneous injection of isoproterenol 85 mg/kg on 29th and 30th days
4	**GTE + ISO**	5	400 mg/kg green tea extract orally once a day for 30 days + subcutaneous injection of isoproterenol 85 mg/kg on 29th and 30th days.
5	**RES + GTE**	5	20 mg/kg resveratrol + 400 mg/kg green tea extract orally once a day for 30 days
6	**RES + GTE + ISO**	5	20 mg/kg resveratrol + 400 mg/kg green tea extract orally once a day for 30 days + subcutaneous injection of isoproterenol 85 mg/kg on 29th and 30th days

Animals were sacrificed on 31st days after 24 hrs of the last dose using ether anesthesia. Hearts were removed and weighed. A thin slice was taken from each hearts for histopathological [21] and infarct size estimations [22].

2.1. Statistical analysis:

Experimental data were expressed as mean ± SEM and compared by one-way analysis of variance (ANOVA) with post hoc analysis. The tukey-kramer post hoc test was applied to identify significance among groups. $P < 0.05$ was considered as statistically significant. Graph Pad Prism 3.0 software, Inc. (version 3.06) was used for statistical analysis.

3. RESULTS:

3.1. Histopathological studies:

After sacrificing the animals on 31st day, hearts were removed, washed in ice cold normal saline and preserved in 10% formalin. Tissues were then stained by eosin-haematoxylin stains and the sections were studied to determine the extent of tissue damage by the free radicals and recovery by the test drugs.

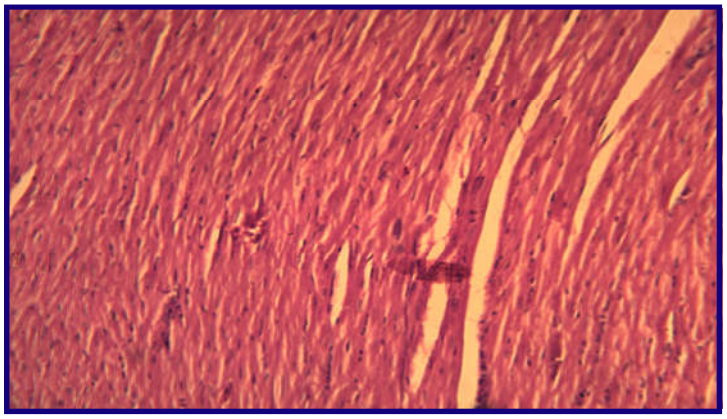


Figure 1. Photomicrograph of normal control group showing normal architecture of myocardium with no infiltration and vacuolation of cells.

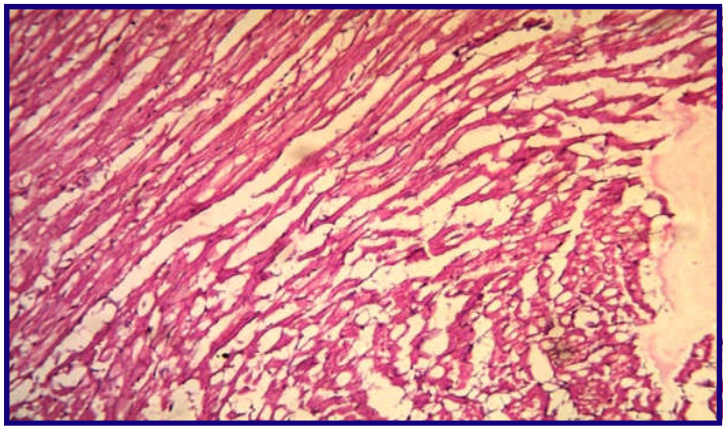


Figure 2. Photomicrograph of (ISO) isoproterenol group showing remarkable disintegration of myocardial muscle fibers, vacuolation and the presence of pyknotic nucleus.

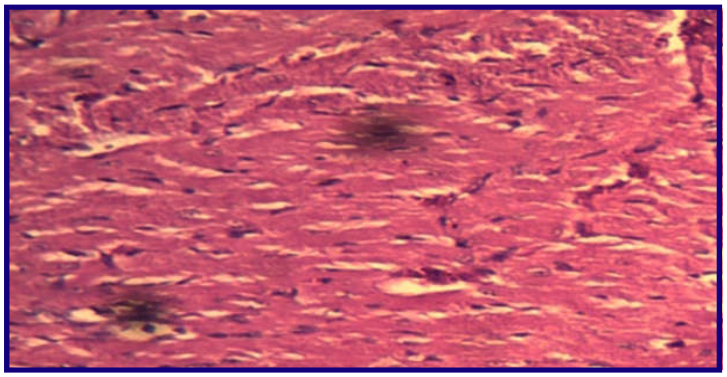


Figure 3. Photomicrograph of (RES + ISO) Resveratrol treated group showing almost normal architecture of myocardial fibers.

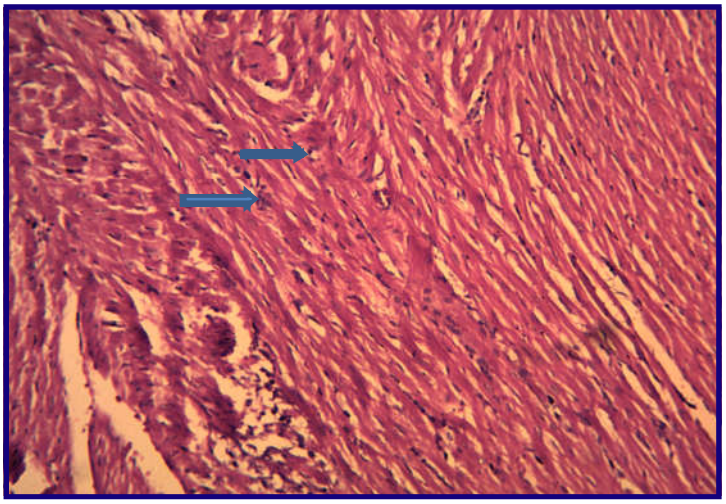


Figure 4. Photomicrograph of (GTE + ISO) green tea extract treated groups showing few pyknotic nucleus and vacuoles.

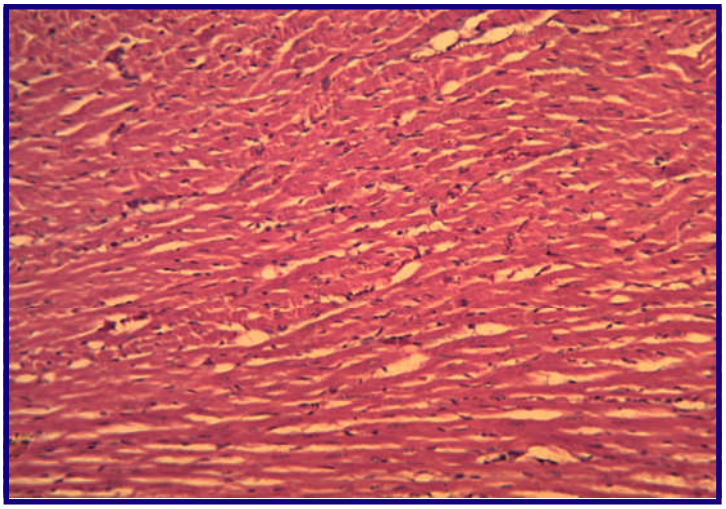


Figure 5. Photomicrograph of (RES + GTE +ISO) combination treatment group showing almost normal cellular architecture.

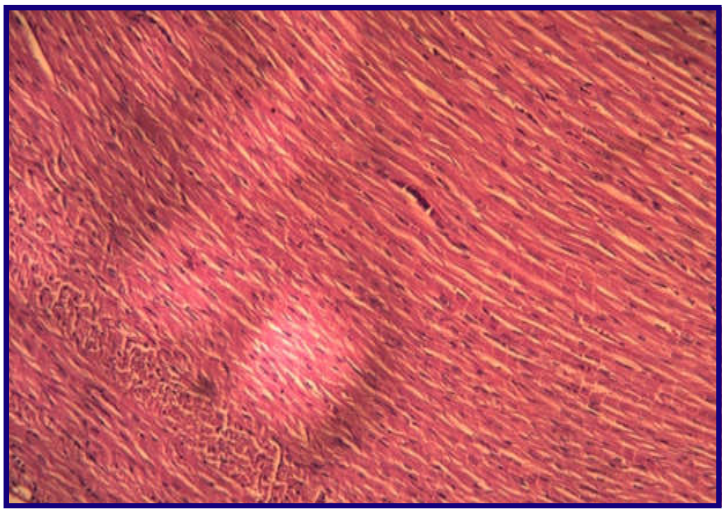


Figure 6. Photomicrograph of (RES + GTE) *Per se* group showing normal cellular architecture similar to control group.

3.2. Estimation of infarct size in heart tissue.

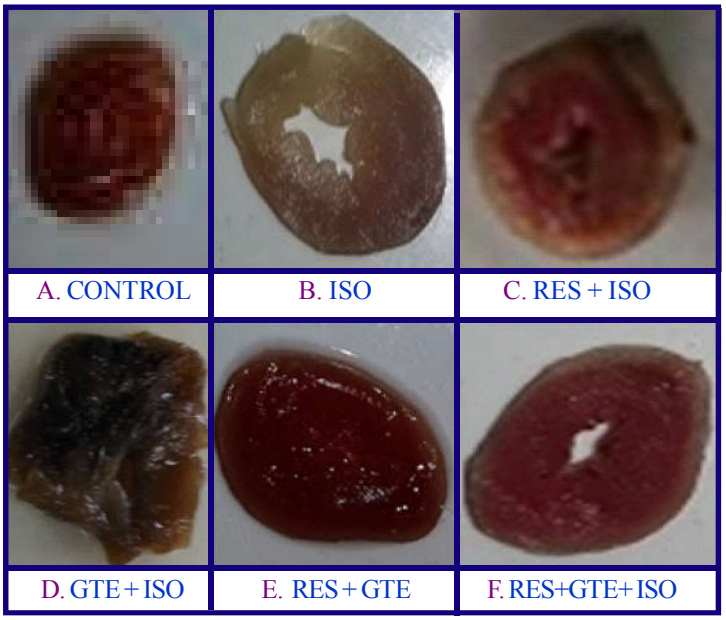
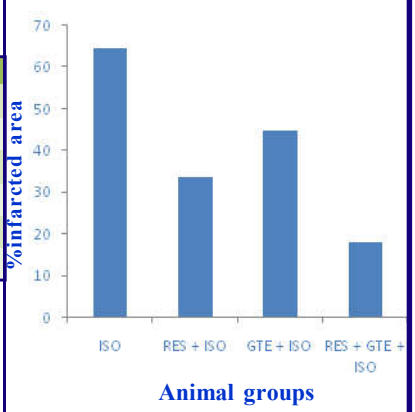


Figure 7. Photographs of tetrazolium treated heart sections. White colored part of the heart tissue is showing Infracted area and red as normal living cells. A. Control; B. ISO, isoproterenol (85 mg/kg s.c); C. RES + ISO, resveratrol (20 mg/kg orally) + Isoproterenol (85 mg/kg s.c); D. GTE + ISO, green tea extract (400 mg/kg orally) + isoproterenol (85 mg/kg s.c); E. RES + GTE, resveratrol (20 mg/kg orally) + green tea extract (400 mg/kg orally); F. RES + GTE+ ISO, resveratrol (20 mg/kg orally) + green tea extract (400 mg/kg orally) + Isoproterenol (85 mg/kg s.c).

Table 1: Percentage infarct size of different groups.

Groups	% infarct size
CONTROL	No infarction
ISO	64.47
RES + ISO	33.7
GTE + ISO	44.78
RES + GTE+ ISO	17.92
RES + GTE	No infarction



Graphical Representation of Table 1.

4. DISCUSSION

In the present study, we have tried to evaluate the cardioprotective role of resveratrol and green tea extract in combination on the basis of histopathological changes and extent of infarction produced by isoproterenol in Wistar rats. Development of cardiac dysfunction is generally related to chronic β-adrenergic activation in the heart cell[23] that leads to an increased oxidative stress due to an increase in cardiac reactive oxygen species (ROS) production and decrease in

antioxidant capacity [24]. It has been further reported that free radicals and oxidative stress are the common mediators of apoptosis and necrosis directly or via lipid peroxidation [25]. ISO depletes the energy reserve of cardiac cells thereby a complex biochemical and structural changes occur that causes cell damage, which leads to necrosis [26]. In this study we found that resveratrol, green tea extract and their combination have shown significant restoration of cellular damage and considerable decline in myocardial infarct size which goes fine with the earlier findings [27]. Control group animals showed normal architecture with cellular integrity of myocardium. However isoproterenol administration caused myocardial edema, increased myofibril thickness and pyknotic nucleus. Resveratrol, green tea and their combination reverted these changes and manifested normal architecture. Although resveratrol, green tea extract and their combination all showed cardioprotection in terms of histopathological examinations and infarct size calculation, but the protection offered by the combination of resveratrol and green tea extract was significantly higher than the two drugs alone. The protection shown by these drugs is well attributed to the established fact that resveratrol and green tea extract counters oxidative stress by maintaining antioxidant enzyme and cellular integrity. In addition, resveratrol and green tea extract combination can be considered as a better option for a huge population suffering from cardiac and other life style related disease rather than treated individually. Both the drugs are herbal nutritional supplement having negligible side effects, easily available, economic and show wide range of health benefits.

5. CONCLUSION:

Resveratrol and green tea extract have shown protection against isoproterenol induced myocardial infarction. Resveratrol has shown better cardio protection than green tea extract. However their combination proved to be a better option.

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